

## **AMENDMENTS TO THE SPECIFICATION**

Please replace the paragraph bridging pages 15 and 16 of the specification with the following corrected paragraph:

Mutagenic plasmid pREG104 was constructed for unmarked gene deletion of *kstR*, the gene encoding a transcription regulator of the *kstD* gene (encoding 3-ketosteroid  $\Delta^1$ -dehydrogenase KSTD1) in *Rhodococcus erythropolis* SQ1 (Fig. 1). Briefly, pSDH205 (Van der Geize, R. *et al.* 2000. Appl. Environ. Microbiol. 66:2029-2036) was digested with restriction enzymes *Nru*I and *Bal*II, followed by self-ligation, resulting in plasmid pREG103. An *Eco*RI DNA fragment of pREG103, containing the *kstR* gene deletion was subsequently cloned into *Eco*RI digested pK18mobsacB vector, resulting in pREG104. Unmarked *kstR* gene deletion mutant *R. erythropolis* RG10 was isolated from *R. erythropolis* SQ1 using pREG104 via the sacB counter-selection method as described (Van der Geize R. *et al.* 2001. FEMS Microbiol. Lett. 205:197-202). Genuine *kstR* gene deletion was confirmed by the polymerase chain reaction (PCR) using forward primer (REG-FOR) 5'GGCGACGTTGCCGAGAATT 3' (SEQ ID NO:4) and reverse primer (REG-REV) 5'TCAGTGTCGTGAGAGATTCA 3'(SEQ ID NO:7). A PCR amplicon of 618 bp was obtained with parent strain SQ1 genomic DNA (control). With genomic DNA of *kstR* gene deletion mutant strain RG10 the amplicon was reduced to 393 bp, confirming *kstR* gene deletion.

Please replace the paragraph bridging pages 17 and 18 of the specification with the following corrected paragraph:

A *Rhodococcus* expression vector was constructed for the expression of genes under control of the *kstD* promoter of *R. erythropolis* SQ1 (Van der Geize, R. *et al.* 2000. Appl. Environ. Microbiol. 66:2029-2036). Using the *kstD* promoter, expression of genes in *R. erythropolis* mutant strains harboring a *kstR* gene deletion will be constitutive due to the absence of the repressor of *kstD* expression. The *kstD* promoter region (158 bp) was isolated from *R. erythropolis* SQ1 chromosomal DNA by PCR amplification (25 cycles: 30s 95°C, 30s 64°C, 30s 72°C, using *Taq* polymerase) using forward primer

5'ATAAAGCTTATCGATTATGTGTCCCGGCCGCGAAC3' (SEQ ID NO:8) and reverse primer 5'ATAGGTACCATATGTGCGTCCTTACTCCAAGAGGG 3' (SEQ ID NO:9). A *NdeI* site (underlined) was incorporated in the amplicon to be able to clone genes of interest precisely at the ATG startcodon of the *kstD* gene. The amplicon (175 bp) was blunt-ligated into the unique *SnaBI* restriction site of shuttle vector pRESQ (Van der Geize, R. *et al.* 2002. Mol. Microbiol. 45:1007-1018) and the resulting Rhodococcus expression vector was designated pRESX (Fig. 2).

Please replace the first full paragraph on page 18 of the specification, starting at line 5, with the corrected paragraph as follows:

The *kstD2* gene, encoding the KSTD2 isoenzyme in *R. erythropolis* SQ1, was isolated from chromosomal DNA of parent strain SQ1 by PCR (conditions: see above), using forward primer 5' GCGCATATGGCTAAGAATCAGGCACCC 3' (SEQ ID NO:10) (*NdeI* site underlined) and reverse primer 5' GCGGGATCCCTACTTCTCTGCTGCGTGATG 3' (SEQ ID NO:11) (*BamHI* site underlined). The introduced *NdeI* and *BamHI* sites were used to ligate the *kstD2* amplicon into *NdeI/BglIII* digested pRESX vector. The resulting plasmid was designated pRESX-KSTD2.

Please replace the third full paragraph on page 19 of the specification, starting at line 17, with the corrected paragraph as follows:

The *kshA2* gene was placed under control of the *kstD* promoter in pRESX. In order to achieve this, the *kshA2* gene was amplified from *R. erythropolis* chromosomal DNA as template by PCR using forward primer 5'GGCCATATGTTGACCACAGACGTGACGACC 3' (SEQ ID NO:12) (*NdeI* site underlined) and reverse primer 5'GCCACTAGTTCACTGCGCTGCTCCTGCACG 3' (SEQ ID NO:13) (*SpeI* site underlined). The obtained *kshA2* amplicon was first ligated into *EcoRV* digested pBlueScript (II)KS (pKSH311) and subsequently subcloned as a *NdeI* / *SpeI* fragment into *NdeI* / *SpeI* digested pRESX, resulting in pKSH312.